Determining the Relative Orientations of Single-shot Diffraction Patterns from Individual Macromolecules and Nanoparticles

Ravi Shankar Vaidyanathan, Valentin Shneerson, Dilano Saldin, and *Abbas Ourmazd*

Department of Physics, University of Wisconsin-Milwaukee

In about an hour, 10^5 "shots" from the LCLS XFEL should scatter $\sim 10^3$ photons into each pixel of the high-q regions of a diffraction pattern from a 500 kDa biological molecule - if only we could integrate the scattered photons from successive shots. In the absence of orientational alignment, each single-shot diffraction pattern will emanate from an unknown random orientation of the molecule. A key problem therefore concerns determining the relative orientations of the individual diffraction patterns in the presence of noise; a "high-q pixel" of a single-shot diffraction pattern is expected to contain an average of $\sim 10^{-2}$ photons.

This presentation addresses the critical issue of finding the relative orientations of individual diffraction patterns at low signal-to-noise ratios in the absence of molecular orientational order.

We have developed and tested an algorithm that can reliably reconstruct the 3D electron density of a molecule from a set of ideal (noise-free) diffraction patterns, each emanating from an unknown random orientation of the molecule, and investigated its performance in the presence of shot noise, modeled by a Poisson distribution. A modified implementation of the algorithm to orient diffraction patterns by the so-called common-line method used in 3D electron microscopy functions satisfactorily for average signal levels as low as ~ 10 photons/pixel, excluding a (high-intensity) central region subtending a semi-angle of 6° around the undiffracted beam. This performance is comparable with that achieved in 3D-EM, where starting images (which contain phase information) have typical S/N ratios of $\sim \sqrt{10}$ [1]. However, this is still ~ 3 orders of magnitude away from that required to orient single-shot XFEL diffraction patterns from single biological molecules.

The noise robustness can, in principle, be further improved by schemes, such as Principal Components or Correspondence Analysis, which consider the totality of the diffracted data without any knowledge of underlying correlations. Experience in other fields has shown that information theoretic limits can be approached by algorithms which explicitly consider inherent correlations within the entire data set, using physically based models.

We will describe the capabilities and limitations of each approach for practical orientation of individual diffraction patterns and structure recovery in the presence of severe signal-to-noise limitations. This is an essential pre-requisite to any experimental determination of single-molecule structures in the absence of molecular orientational order.

[1] Frank, J., *Three-Dimensional Electron Microscopy of Macromolecular Assemblies* (Oxford University Press, 2006)